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BIOLOGY.—*A consideration of mineral nutrition of boxwood in relation to infection by meadow nematodes*, Pratylenchus spp.[1]

A. C. Tarjan, University of Maryland. (Communicated by G. Steiner.)

Plant nematology is a comparatively recent science. To date, most of the investigations in this field have been concerned mainly with a study of the pathogenic nematodes, the diseases they cause, and possible means of control of these diseases. A few German workers (*9*, *15*, *17*) have conducted chemical analyses of infected plant parts, but these have been principally on plants infected by the sugar-beet nematode, *Heterodera schachtii* Schmidt, while most of the other nematode-induced plant diseases have not yet been studied as to mineral requirements of the plant hosts.

Certain meadow nematodes, members of the genus *Pratylenchus* attacking the roots of boxwoods, can cause a pronounced decline of these plants (*13*). Symptoms appearing on the above-ground parts are described as an

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unhealthy appearance of the plants resulting from partial to almost complete defoliation, unnatural reddening or chlorosis of the leaves, and stunted growth. An inspection of the root system shows a dwarfed bushy type of growth with a pronounced absence of deep feeding roots. A close observation will reveal brown to black lesions, which usually girdle the roots and eventually cause a sloughing off of the cortical tissues, leaving the fibrous vascular cylinder exposed. It is therefore quite apparent that any plant thus impaired can not long survive without soon indicating in the above-ground parts the deteriorated condition of the root system. Similar symptoms have been observed in the case of other plant nematode diseases. Shaw (*11*) states that the foliage of beet plants, whose roots are invaded by the sugar beet nematode, assumes a lighter green color than normal, with subsequent more severe discoloration of the foliage and death of the plant. Infected plants appear to have an abnormally large number of lateral rootlets. These observations parallel those of Tyler (*14*) who worked with the root-knot nematode, Cobb (*4*) who investigated the activities of the burrowing nematode, *Tylenchus similis* Cobb, and Steiner (*12*) who described symptoms caused by various members of the genus *Pratylenchus* on several types of plants.

On the basis of the symptoms on boxwood foliage described above, it may be suspected that infected plants are suffering from deficiencies of essential elements. The American Plant Food Council (*1*) states that nitrogen deficiency causes a sickly, yellow-green color with a slow, dwarfed mode of growth of the plant. Potash deficiency manifests itself in a mottling, spotting, or streaking of the leaves, while magnesium deficiency results in a loss of green color with definite and sharply defined yellowish-green streaks throughout the leaf. Almost all these symptoms are noticeable in the early stages of the boxwood nematode disease. Kramer and Schrader (*6*) found, in a study on the deficiency symptoms on the Cabot variety of *Vaccinium corymbosum* L., that a magnesium deficiency caused one sharply defined symptom in particular: the leaf margins became chlorotic while the tissue near the midrib remained green. A secondary stage of the deficiency resulted in the chlorotic areas turning red and necrotic. A similar condition is found in infected boxwood leaves and has been, in the past, attributed to either drought or winter injury. In a series of tests by Keyes (*5*) on *Gardenia veitchii* Hort., it was found that a potassium deficiency caused chlorosis of the leaves as well as a cessation of growth; further, that calcium deficiency resulted in a breakdown of root tissue starting at the tips of the roots. New roots were not produced and as the disintegration of the root system progressed, wilting occurred. Although in nematode-infected boxwood roots there occurs a breakdown of the roots inhabited by nematode colonies, this has been attributed to the destruction of the cortical tissues by the nemas which are usually situated between the healthy and deteriorating portions of the rootlet.

Vaňha (*15*) analysed the dry matter of the roots of nematode-infected sugar beets and found lower percentages of phosphorus, magnesium, and calcium and a decidedly lower percentage of potassium in the infected roots in contrast to the healthy. Wilfarth and Wimmer (*17*) likewise found that infected sugar beets had lower percentages of nitrogen, potassium, sodium, calcium, and magnesium. They concluded that the plants were deprived of nutritional substances due to the presence of the nematodes, and stipulated that an abundant potassium fertilization would maintain the proper sugar content within the beets but would not prevent a lowering of the yield. They assumed that proper yields could be maintained only by a comprehensive and abundant fertilization program. Roemer (*9*), however, was able to increase the yield of nematode-infected sugar beets from 18,000 to 19,000 kg per 2.47 acres by applying only a potassium fertilizer.

It can therefore be seen that in the case of certain plant nematode diseases there is a marked reduction in certain of the nutritional elements contained in the plants. The present investigations were undertaken with the objective of correlating the disease symptoms expressed by boxwood plants infected by meadow nematodes with a possible excess or deficiency in the amounts of some of the elements required for normal growth by the plants.

MATERIALS AND METHODS[2]

A total of 48 6-month-old dwarf English boxwoods, *Buxus sempervirens* var. *suffruticosa* L., which had been rooted and grown under comparable conditions, were transplanted into a potting medium of approximately one-third sand and two-thirds soil in 5-inch pots. These were separated into two groups of 24 plants each. One and one-half grams of finely chopped, healthy boxwood roots were incorporated into the soil in each pot in one group, while 1½ grams of finely chopped meadow nematode-infected boxwood roots were applied to the soil in each pot in the other group.

After six months of growth, all plants were washed and weighed. The mean weight per plant in each group is presented in Table 1. The plants were then dried for 48 hours at approximately 125°F. The 24 plants in each group were then separated into six lots of four plants each, and each lot was further separated into stem, leaf, and root samples. These were ground in a Wiley Mill equipped with a 60-mesh wire screen.

Ash solutions were prepared and analyzed for nitrogen, phosphorus, and magnesium by methods suggested by Lindner (*7*). In the preparation of reagents for the determination of nitrogen, Nessler's solution was prepared according to Vanselow (*16*).

Potassium, sodium, and calcium readings were obtained by use of the Beckman flame spectrophotometer.

Boron was determined by the same methods proposed by Berger and Truog (*2, 3*) except that the color comparisons were made by use of a Coleman spectrophotometer at a wavelength of 610 millimicrons (*10*).

Table 1.—Weight Comparisons between Healthy and Meadow Nematode-infected Boxwoods.

	Healthy	Diseased
Weight in grams	18.6* ± 1.2	14.6† ± 0.7

* L.S.D. at the 1-percent level is 2.6.
† Each value represents the mean of 24 replicate plants.

RESULTS

As can be seen in Table 2, the amount of calcium in the diseased stem, leaf, and root samples was higher than in the healthy samples. This is contrary to the results of Vaňha (*15*) and Wilfarth and Wimmer (*17*), who found, in working with sugar beets infected with the sugar-beet nematode, that there was a lower amount of calcium in the diseased tissues than in the healthy.

The differences in boron content between healthy and diseased samples were insignificant.

Magnesium was higher in healthy stems, diseased leaves, and healthy roots. Other investigators (*15, 17*) had not found differences in magnesium (in oxide form) to be very great in analysis of healthy and diseased sugar beet plant parts.

Sodium was higher in diseased stem and leaf samples while in root samples it was higher at the 1-percent level of significance.

Nitrogen was higher in healthy stem and leaf samples while in root samples it was

[2] The author expresses sincere appreciation to Dr. L. E. Scott, Department of Horticulture, University of Maryland, for his valuable suggestions, guidance, and criticisms during the execution of the chemical determinations involved.

Table 2.—Mineral Content of Healthy and Diseased Boxwoods Expressed in Milligrams per Gram of Oven-dry Sample

Mineral	Stems		Leaves		Roots	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Boron	0.005* ± 0.002	0.007 ± 0.002	0.036 ± 0.006	0.043 ± 0.008	0.002 ± 0.001	0.007 ± 0.005
Calcium	0.48 ± 0.13	0.83 ± 0.15	1.53 ± 0.11	1.88 ± 0.18	0.35 ± 0.05	1.02 ± 0.16
Magnesium	5.83 ± 0.88	4.92 ± 1.00	15.67 ± 0.38	16.75 ± 0.79	7.42 ± 0.93	6.08 ± 0.79
Nitrogen	22.60 ± 2.65	21.10 ± 0.54	41.50 ± 0.74	40.20 ± 0.69	37.20 ± 1.31	47.60† ± 1.67
Phosphorus	1.50 ± 0.22	1.70 ± 0.16	2.70 ± 0.10	3.70† ± 0.27	2.89 ± 0.26	3.20 ± 0.14
Potassium	2.10 ± 0.03	2.07 ± 0.14	5.43† ± 0.32	1.88 ± 0.18	5.00† ± 0.18	3.33 ± 0.11
Sodium	0.50 ± 0.07	0.82 ± 0.54	0.68 ± 0.05	0.82 ± 0.05	0.48 ± 0.05	1.03† ± 0.16

* Each value represents the mean of six composite samples.
† Significant at odds of 99 to 1.

significantly higher at the 1-percent level in diseased samples as compared to healthy samples.

Phosphorus, as in the case of nitrogen, was higher in diseased stem and root samples than in healthy samples and was significantly higher at the 1-percent level in diseased leaves than in healthy leaves.

Potassium in healthy stem samples was higher than in diseased stem samples, while it was significantly higher at the 1-percent level in both the leaf and root samples as compared to the diseased.

DISCUSSION

The results presented above appear to be due to the deleterious effects of feeding of meadow nematodes on the roots of box wood plants. It may be that the gradual rotting away of the root system due to the nematodes is cardinal in instigating the decline and eventual death of the plants, but here one would assume a proportionate decrease in the level of all minerals contained in the stems and leaves rather than a decrease in some and an increase in others as occurred. Then too, one should not preclude the fact that secondary invaders, which are in constant association with the nematodes, may be the real pathogens which cause the decline of these plants. Since Koch's postulates can not be proved for this disease, as well as for diseases caused by viruses or by other obligate parasites, we can not definitely assert that the meadow nematode is the primary cause of decline in boxwood, but must be content with stating that this nematode is almost constantly associated with this disease.

Another very important possible cause of decline in boxwood is brought to light by the work of Linford (*8*) who found that various types of nematodes, such as *Aphelenchoides* spp., *Ditylenchus* spp., and *Aphelenchus* spp., have esophageal glands that are salivary in nature. He observed that the above-named nemas ejected fluid from their esophageal glands into the root tissues of the host while feeding. These fluids, which are strongly digestive in nature, functioned in the breaking down and digestion of the tissues and were then sucked back into the nematode body. Linford concluded that "destructiveness may be closely related to the volume and composition of the saliva injected into the tissues, rather than to the ability of the parasite to destroy tissue by mechanical means." Following Linford's reasoning, we find that inspection of the meadow nematode microscopically reveals three rather large esophageal glands, which undoubtedly play an important role in the feeding of this parasite. It may actually be then that the symptoms of this disease are, for the most part, a response of the plant to the esophageal secretions ejected by the nematodes, and that these secretions are toxic to the plant.

In view of the above findings, a method is suggested of possibly alleviating the effects of this disease and allowing the affected plants to regain, at least in part, some of their former vigor. Infected boxwoods could be given fertilizers containing relatively heavy concentrations of potassium and low concentrations of sodium, nitrogen, and phosphorus. These materials could either be broadcast directly on the soil, applied to the soil as a liquid fertilizer, or injected into the plants. In theory, the restoration of the normal balance of mineral nutrients in the plant would promote a refunctioning of the normal plant metabolism, and new plant parts would eventually be produced. This might improve the appearance of the plants considerably but would not necessarily indicate that they were becoming immune to the effects of feeding by the nematodes. Rather, it would add to the life of the plants, and possibly insure their successful competition with the nematodes until some effective methods of control were devised.

SUMMARY

Mineral analyses for boron, calcium, magnesium, nitrogen, phosphorus, potassium, and sodium were conducted using stems, leaves, and roots of healthy and meadow nematode-infected dwarf English boxwoods, *Buxus sempervirens* var. *suffruticosa*. It was found that roots of infected plants contained higher levels of sodium and of nitrogen whereas the roots of healthy plants contained a higher level of potassium. Leaves of infected plants contained a higher level of phosphorus, while leaves of healthy plants contained a higher level of potassium.

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ENTOMOLOGY.—*A new genus and three new species of Microlepidoptera from California (Ethmiidae).*[1] J. F. Gates Clarke, Bureau of Entomology and Plant Quarantine.

The following new species and new genus are described to provide names to correspondents and to continue the arrangement and development of the National Collection.

Ethmia nadia, n. sp.

Figs. 1-1a

Alar expanse, 20–22 mm.

Labial palpus sooty black; basal segment heavily white scaled, second segment sparsely so and a narrow apical white annulus and the third segment white scaled above. Antenna sooty black with sparse white scaling on scape basally. Head sooty black with white scaling between the antennae and laterally. Thorax sordid white with four sooty-black spots placed one on each side, one anteriorly and one posteriorly; tegula sordid white with a black spot basally. Fore and hind wings blackish brown, cilia concolorous; forewing with a longitudinal white streak extending from base to end of cell where it forks, the scaling bordering the fork, black; the costal edge of the longitudinal streak narrowly edged with black, the dorsal portion overlaid with gray; at basal fifth, in the longitudinal streak, a black spot; around termen a series of indistinct black spots. Forelegs and midlegs blackish brown; hindlegs ocherous. Abdomen, except blackish brown first segment, orange-ocherous.

Male genitalia.—As figured. The posterior edge of gnathos with a strong comb of teeth; anterior portion with strongly sclerotized lateral teeth.

Female genitalia.—Female unknown.

Type.—U.S.N.M. no. 59367.

Type locality.—McCloud, Calif.

Remarks.—Described from the type ♂ and two ♂ paratypes, all from the same locality (June 5, 1935, E. C. Johnston). Both paratypes in Mr. Johnston's collection.

This species is nearest to *E. albistrigella* (Walsingham) but differs from it by the wider basal portion of the longitudinal streak of the forewing and by the presence of the black spot, in the white streak, at basal fifth. The anterior portion of the gnathos of *albistrigella* is studded with small teeth which are absent in *nadia*.

[1] Received March 14, 1950.